THE SALIVARY GLAND CHROMOSOMES OF *CULEX TARSALIS*

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ABSTRACT. The salivary gland chromosomes of *Culex tarsalis* are described and briefly compared to similar descriptions of other *Culex* species previously published. Additional cytogenetic evidence will be needed to make meaningful comparisons and hypothesize speciation relationships.

INTRODUCTION

Important advances in genetics have been made through studies on the polytene chromosomes of Diptera, primarily *Drosophila* species. These giant chromosomes are usually 100–200 times larger than the mitotic chromosomes. In addition, their distinctive banding patterns facilitate recognition of aberrant, alternate or polymorphic arrangements as well as identification of specific sections relative to morphological and/or physiological mutations.

The most extensive studies with mosquito polytenes have been made among the anophelines, since preparations of these giant chromosomes, both from salivary glands and ovarian nurse cells, can be routinely obtained. Coluzzi and Kitzmiller (1975) and Green (1981) give good reviews of anopheline cytogenetics.

In the culicine subfamily, which includes the medically important *Culex* and *Aedes* genera, the preparation of polytenes has been more difficult, primarily because of greater length and poor spreading due to intra- and interchromosomal connectives (Amirkhanian 1974, Kitzmiller 1976). Thus most cytogenetic studies have been limited to mitotic and meiotic configurations.

Kitzmiller and Keppler (1961) briefly described some details of the salivary chromosomes of *Culex pipiens* Linn. However, the breakthrough in polytene studies for the Culicidae came with Dennhofer's publication of the *Cx. pipiens* map in 1968. Sharma et al. (1969) and Kanda (1970) broadened the cytogenetic base of the *Cx. pipiens* complex with a map of *Cx. quinquefasciatus* Say, and Amirkhanian (1974) further expanded information on the complex with a map of the Tehran strain of *Cx. pipiens* [*molestus*]. Tewfik and Barr (1974) suggested there were no major chromosomal differences in what were formerly considered as the various *Cx. pipiens* subspecies, and attributed minor differences to preparation techniques. More recent *Culex* polytene studies included *Cx. gelidus* Theobald (Pasahan 1980) and *Cx. vishnui* Theobald (Chaudhry 1981).

Culex tarsalis Coquillett is of medical importance as the primary vector of western equine encephalomyelitis and St. Louis encephalitis in western North America. Extensive genetic and cytogenetic studies have been pursued with this species, primarily in relation to the possibility of using genetics in control programs (Asman et al. 1982, McDonald and Asman 1982). As in other Culicidae, the chromosome number is 2n = 6, with no distinguishable differences in the karyotypes of the 2 sexes (Asman 1974). Rather, sex determination depends on a single gene or short segment of a chromosome.

This paper presents the first known description of the salivary-gland polytenes of *Cx. tarsalis* and briefly compares them with those of other *Culex* species. It represents a study of over 200 acceptable preparations of salivary-gland nuclei using currently known techniques and over 300 photographs of distinct sections. It will, hopefully, be of value in further elucidating the cytogenetics and genetics of this species.

MATERIALS AND METHODS

A Bakersfield, CA field-derived, laboratory strain was used for these studies. First-instars were reared 100 per pan in 1,500 cc water under standard rearing procedures (Asman et al. 1983). Early 4th instars with minimal quantities of fat bodies were used for dissection since they gave the best salivary glands for slide preparation.

The methods of dissection and slide preparation described by Amirkhanian (1974) were used with modifications of time, sequence of fixations and stain substitution. Salivary glands were dissected in 65% saline, then placed in a 1:1 combined solution of Carnoy's fixative and Battaglia's (1959) hydrolyzing fluid for 90 seconds on siliconized slides. After blotting off the liquid, French's dissecting fluid (French et al. 1962) was added for 12–13 minutes. Following the removal of the latter, the glands were stained with a drop of 1% lacto-aceto-orcein as described by Breland (1961) for 2 minutes. Thereafter a clean coverslip was gently applied. To aid with the spreading of the chromosomes, the coverslip was lightly tapped with the head of a pencil prior to applying stronger pressure with the thumb to the slide now enfolded in a piece of filter paper. Chromosomes were pho-

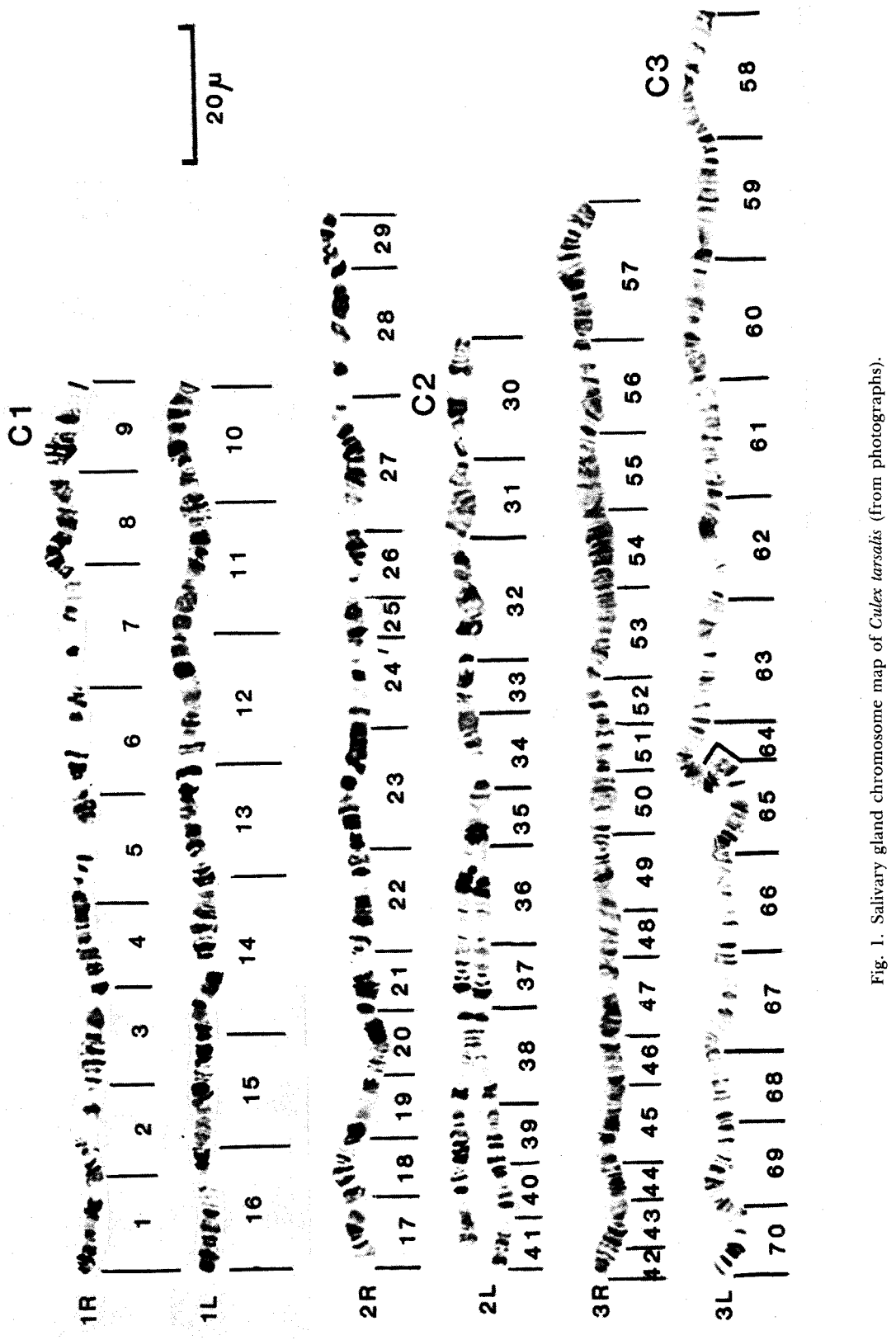

Fig. 1. Salivary gland chromosome map of *Culex tarsalis* (from photographs).

tographed 40X magnification using an Olympus PM-6 camera mounted on a Tiyoda R21 microscope and Panatomic-x film.

RESULTS AND DISCUSSION

The salivary gland nuclei of *Cx. tarsalis* consist of 3 pairs of long polytenic chromosomes with distinct banding patterns (Fig. 1). Asynapsis in all pairs appeared to be common and was presumed to occur randomly at various regions including the centromere. This phenomenon, which can be seen in the centromere as well as in zones 36–41 of chromosome 2 (Fig. 1), is thought to be due to the position of the chromosomes on the slide and/or to the amount of pressure applied in attempting to spread the 3 pairs. While interchromosomal connective fibers consistently limited good spreading along the main axis of each pair, the terminal ends were commonly free and easy to identify. This differs from *Cx. pipiens* as reported by Kanda (1970) and Amirkhanian (1974) where free ends were rarely seen.

The centromeres of *Cx. tarsalis* are distinct; however, they were never seen as the large spherical puffs described for *Cx. pipiens* salivary-gland chromosomes (Dennhofer 1968, Kanda 1970, Armirkhanian 1974, Tewfik and Barr 1974). In *Cx. tarsalis* the centromeres are only modestly enlarged (Fig. 1). Thus the identification of the centromeres in this study was confirmed by their relative position and their lack of stainable chromatin material as noted by Mukherje et al. (1966) and Asman (1974). Similarities in banding patterns bordering the centromeres in *Cx. pipiens* as described by Kanda (1970) also helped to identify the centromeres in *Cx. tarsalis.*

Asman (1974) and Mukherje et al. (1966) both noted, on the basis of mitotic metaphase chromosomes, that numbers 1 and 3 were metacentric while the medium-sized number 2 was slightly submetacentric. Studies on the larger polytenes suggest that number 1 is slightly acentric, number 2 is more acentric, and that the largest, number 3, is even more so.

Following standard procedures for polytene mapping, the chromosomes are divided into 70 zones with each zone subdivided into smaller regions for easier identification (Fig. 2). The right arm of chromosome 1 (1R) contains 8 zones, zone 9 is the centromere, and the left arm (1L) holds zones 10 to 16. The right arm of chromosome 2 (2R) holds zones 17–29, while 2L includes the centromere, zone 30, as well as zones 31–41. The right arm of the longest chromosome (3R) holds zones 42–57, and 3L continues with the centromere, zone 58, and terminates with zones 59–70.

Chromosome 1 is 263 microns in length, number 2 is 296 microns, and number 3 is 368 microns. The ratio of chromosomes 1/2+3 is 0.396 microns, while a similarly estimated ratio for mitotic chromosomes was reported to be 0.296 and 0.297 by Asman (1974) and Mukherje et al. (1966), respectively. This variation can easily be explained by the observation that the polytenes are very flexible—stretching and constrictions are common. Other contributing causes of this difference in length relationships could well be methods of chromosome preparations, use of different strains, and the use of different stages of the immatures,—larvae versus young pupae, respectively. Table 1 compares the lengths of *Cx. tarsalis* salivary-gland polytenes to those of other *Culex* species. In all 3 pairs of polytenes, the centromere region in *Cx. tarsalis* is at the lower end of the length range for *Culex* species.

The range of lengths of the chromosomes among different species and subspecies is rather broad (Table 1). At this point, study of the banding similarities of individual zones would be more valid in attempting to assess intragenera relationships.

It is assumed from the many slide preparations that in some distinct areas the swelling or bulbs and the restrictions are permanent landmarks, and therefore can serve as points of identification in addition to the banding patterns; however, additional studies will be needed to clarify these assumptions. As stated earlier, good sections of the 3 chromosome pairs of *Cx. tarsalis* were not easily or routinely obtained, and while many preparations were studied, the banding patterns described below could easily be somewhat modified in the future, especially as improved techniques for obtaining polytenes in *Cx. tarsalis* are developed.

Judging from the limited, although growing, literature on Culicidae cytogenetics, it is obvious that much more cytogenetic evidence is needed on *Cx. tarsalis* as well as on other *Culex* species to help clarify the speciation relationships among *Culex* mosquitoes. Hopefully, this paper will contribute to that body of knowledge.

DESCRIPTION OF THE CHROMOSOMES

Chromosome 1. The right arm starts as a bulb beginning with a dark heavy band, followed by a thin band, then a broader heavier one (A). Subunit B consists of 2 moderately-sized bands, and C has 2 narrow and 2 broader bands. Zone 2A holds 3 medium bands and a wider band, B consists of an achromatic area followed by granular lines, a broad band and a narrow one. Zone 3 holds a broad band, a thin one, 2 broad lines and a narrow one (A); sub-

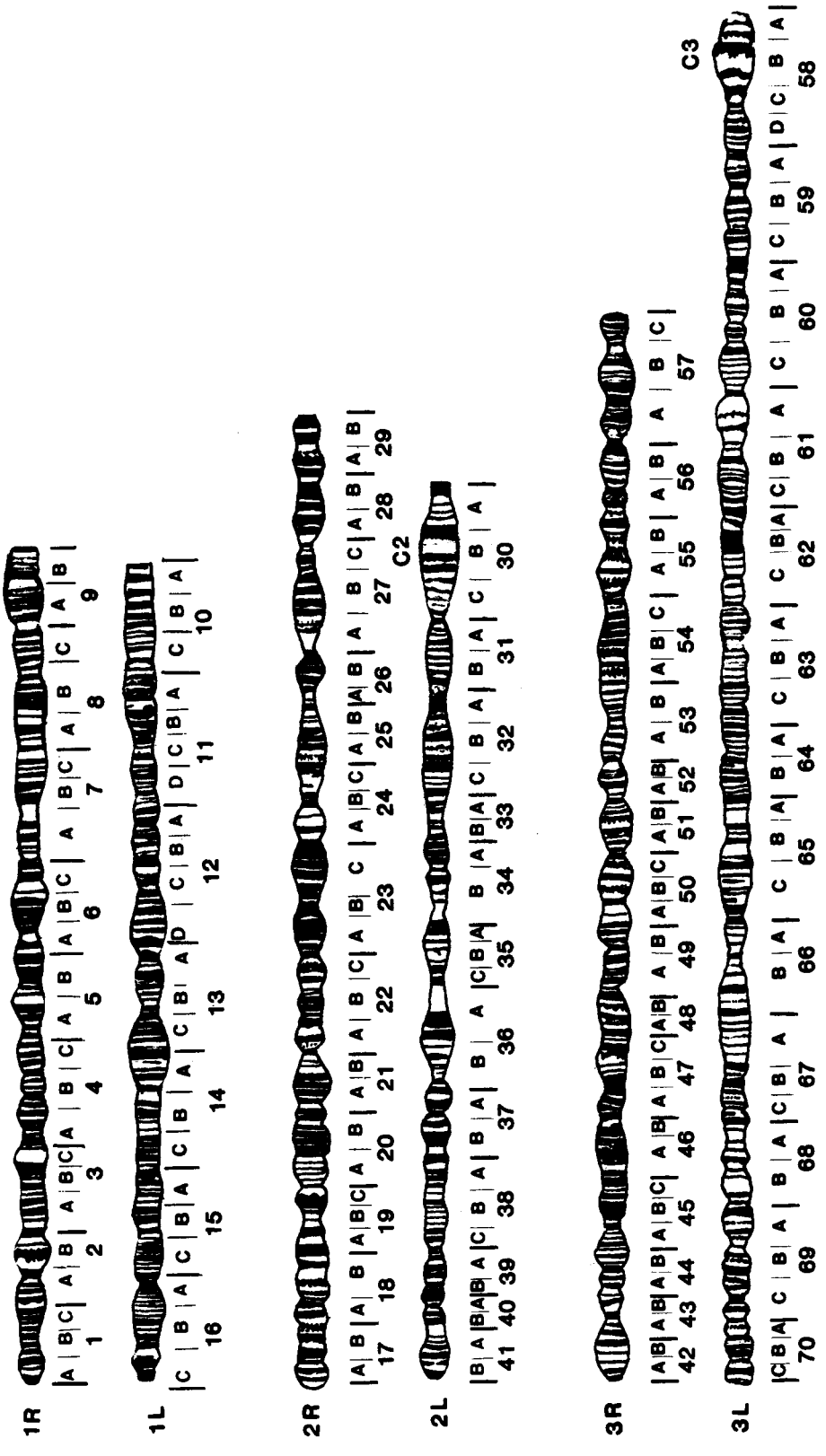

Fig. 2. Salivary gland chromosome map of *Culex tarsalis*.

Table 1. Salivary gland chromosome size* reported for *Culex* species and *Culex pipiens* complex.

Chromosome	*Culex pipiens* complex						*Cx. vishnui*	*Cx. gelidus*	*Cx. tarsalis*
	pipiens [*molestus*] (Amirkhanian)	*quinquefasciatus* (Sharma)	*quinquefasciatus* (Kanda)	*pipiens* (Dennhofer)	*pipiens* (Kitzmiller)	*pipiens* (Tewfik)	(Chaudhry)	(Pasahan)	(Asman)
I 1R	108	97	140	140	165	119	100	56	123
C-1	13	15	—**	20	20	18	—	19	14
1L	95	82	110	120	150	102	73	75	136
Total	216	194	250	280	335	239	173	150	263
II 2R	199	130	250	250	225	252	121	121	156
C-2	18	18	—	30	20	32	—	20	19
1L	158	125	200	205	170	200	114	124	121
Total	375	273	450	485	415	484	235	265	296
III 3R	191	140	270	275	225	280	141	224	162
C-3	25	20	—	50	30	44	—	36	20
3L	189	162	230	230	200	205	141	171	186
Total	405	322	500	555	455	529	282	431	368

* Microns; ** Centromeres included in arm measurement.

unit B has 2 broad bands separated by a narrow one; and C holds 2 thin bands which border an achromatic area. In zone 4 subunit A has a broad band followed by a light thin line and 3 medium-sized bands; B continues with a medium-sized band, a broader band, 2 narrow ones, and a broader one, and C has 3 similar moderately-sized bands. Zone 5A holds 4 broad bands and an achromatic area, and B has 2 thin light bands in the continued achromatic area followed by a very broad band and a thinner broad one. Zone 6A holds 2 broad bands separated by achromatic material, B follows with 3 broad bands, and C holds achromatic material and 2 moderately-sized bands. In zone 7A the highly achromatic section with 3 thin light bands continues followed by 2 medium-sized bands; B holds 2 broad bands, and C has 3 lightly-stained medium-sized bands. Zone 8 begins with a moderately-sized band, a heavy broad one and a thin light band (A); B follows with 4 broad dark bands and 1 thinner dark band, and C holds 4 moderate bands framed on either side by an achromatic space.

Centromere 1. Zone 9 is somewhat bulbous and holds 5 moderately-sized bands, numbers 2 and 3 of which are more dense (A), while subunit B consists of a broad dark band, 3 thin light bands, ending with a dark but narrow band.

Left arm of chromosome 1: Zone 10 holds 3 dark moderate bands in A; B continues with 3 moderate bands, and C has 4 narrow bands and ends with a broader band. In zone 11, A has 4 moderately-wide bands, B continues with 3 similarily sized bands, C holds a medium, then thin, then moderate band, and D has a series of 8 thin light bands. Zone 12 is also divided into 4 subunits: A holds 4 moderate bands and 1 thin band; B has 2 medium-sized bands, a fine light band followed by a dark broad band; C starts with a granulated achromatic area with 2 medium-sized bands, and D is bulbous with 3 moderate bands in an achromatic area. Zone 13A begins with 2 closely aligned bands, followed by 2 thin lines and a broad band; B follows with a moderate-sized band and 3 narrow bands, and C holds 3 dark moderate bands and 2 closely aligned thin ones. In zone 14, A begins with a conspicuous broad band, a thin band and 3 lighter narrow bands, B holds 2 moderate bands and 3 thin lines, and C holds a series of 5 or 6 medium bands. In zone 15, A has 2 broad bands followed by 3 thinner ones; B consists of 3 broad bands probably made up of a series of fine bands, and C holds 7 or 8 less dense narrow bands. Zone 16 has 2 broad dark and 3 very narrow light bands; B continues with 2 thin, 1 broad, 2 narrow and 2 medium-sized bands, and C consists of 2 broad bands, the terminal one for the chromosome appearing more dense and constricted.

Chromosome 2: Right arm. The tip of the right arm consists of a light bulb followed by 2 dark bands and a constriction holding a narrow band (17A). Two dark broad bands followed by a light granular band are identified as 17B. Zone 18 holds 2 broad bands and 1 narrow band (18A) followed by a medium-sized band, a granular broader band and a dark broad band (18B). Zone 19 has 2 pronounced broad bands as it becomes constricted (19A); 19B is a constricted area with 2 narrow bands, and 19C holds a narrow and a broad band. Zone 20A consists of 4 narrow bands and a fifth broader band, while 5 moderately-sized bands make up

20B. The next section has 4 medium-sized dark bands (21A); 21B has 1 wide dark band and 2 narrow bands as it again becomes constricted. Zone 22 consists of 3 bulbs or puffs; 22A has a light granular band with 2 dark broad lines; 22B holds 1 narrow, 2 broad and 2 narrow bands, and 22C has 2 distinct heavy bands as a bulb. Zone 23 has 5 distinct bands in the first bulb (23A), a constricted area holding 3 bands (23B) and 7 broad to moderate lines in the 3rd bulb (23C). Zone 24 is distinct with an achromatic area (24A) holding a broad and narrow band. This is followed by a small, more constricted area holding 2 distinct bands (24B). The 24C subunit is again highly achromatic with 2 moderately dark and narrow bands. Zone 25 begins with a distinct broad band followed by a granular light area and 2 highly stained distinct bands (25A); 25B has 2 moderately broad bands with 2 light narrow lines between them. Zone 26 begins as a constricted area holding a light and narrow band followed by a heavier one (26A); 26B is a more enlarged bulb holding 4 bands of similar width. Region 27 again begins with a conspicuous achromatic area with some granular narrow lines (27A); 4 broad bands make up the bulb of 27B, while a broad band flanked by a narrow one on the right and 2 on the left make up 27C. Zone 28 begins with a stretched achromatic area and then holds 5 broad bands. In zone 29 (A and B) 4 successive broad bands are conspicuous although the second one appears to be granular rather than solid.

Centromere 2. Zone 30 is recognized by its achromatic material and slightly bulbous shape. The first region begins with a constricted but moderately broad dark band, followed by 4 narrow bands, and ends with a broader band (30A); 30B continues with this broad band, is followed by 1 light thin line, 2 highly broad bands, and ends with a more narrow band as the bulb becomes constricted; 30C again is highly achromatic with 4 narrow bands.

Left arm of chromosome 2: Region 31A holds 3 narrow and 2 slightly broader bands; 31B has 4 narrow bands and 1 moderately broad band. In zone 32, A begins with an achromatic section holding a light band followed by 3 dense narrow bands and 3 light bands. A bulb forms at 32B centrally holding a broad dark band flanked by granular material and ending with a narrow dense band, 32C has a very broad dark section (probably 2 bands), and 2 light bands in a clear area followed by another dense broad band. Zone 33 holds 5 moderately-sized dark bands. Zone 34 begins with 3 broad bands (A) while B is distinguished by a highly achromatic area with a broad band flanked on either side by light narrow bands and ending with another broader line. Zone 35 holds a medium band, 2 light narrow lines in an achromatic region, and 2 dark moderately-wide bands respectively from A to C. Subunit 36A is highly achromatic followed by 3 broad dark bands, and 36B has 4 narrow bands in an achromatic background. Zone 37 consists of 3 light and narrow bands in A, followed by 3 more densely stained lines in B. Zone 38 is recognized by 1 broad band and 3 narrow dark bands in A, 5 narrow light bands in B, and 3 moderately-broad dense bands in C. Zone 39A holds 3 light narrow and 2 medium-sized bands, and B has 2 medium-sized dark lines. Zone 40A has 3 distinct bands, as does 40B, followed by an achromatic region. The terminal bulb (41) holds 6 bands through A and B, increasing in width to the tip.

Chromosome 3: Right arm. The tip of area 42 has 2 distinct moderate bands followed by a narrow band in A, and a narrow and heavier band in B. Zone 43 has 3 medium-sized bands in A with 2 light granular lines in B. Section 44A begins with a faint band followed by 3 distinct bands, while B holds 3 light narrow lines. Zone 45 begins with 5 narrow bands in A, 4 closely-bound narrow dark bands in B, and 4 light narrow lines in C. In area 46A a dark band precedes a granular section, while 46B holds another distinct broad band. Zone 47 begins with 3 broad bands (A), 3 slightly less prominent bands in B, and a single heavier band in a granular-like background in C. Zone 48 contains 4 moderately stained bands through A and B respectively, and area 49 continues with 2 paired sets of narrow dark lines in A and 3 bands of similar width in B. Zone 50A holds a distinct moderate band and then a faint band; B includes a bulb holding a granular section flanked on each side by a moderately-heavy band, while C has 2 light granular lines. Zone 51 has a pair of dark medium-sized bands and a pair of broader but less dense bands in A, followed by 3 light marks in B. Subunit 52A holds a middle-sized band followed by a narrow line and a broad dark band, and B holds a pair of narrow light bands followed by an achromatic area. Zone 53A begins with a dark band followed by a faint narrow line and terminates with 3 more dark bands; 53B follows with 3 moderate bands with a thin line between each. Zone 54A has 2 moderately-sized bands with granulated spaces between them, B has a set of 3 moderate bands, and C also continues with 3, with a space between the last 2. Zone 55 holds 2 narrow dark bands followed by a granulated area, a narrow modestly-dark band and a broader but lighter band (A); subsection B has a second broad but light band, a narrow band of the same intensity, and ends with a dark and

broader band. Subsection 56A starts with an achromatic space, a moderately broad and dark band, and 2 moderately-stained medium-sized bands; 56B holds 2 prominent bands of moderate staining, a very narrow line, and a 3rd band similar to the first 2. It also ends with a moderately dark band at a point of constriction. Zone 57 has a series of indistinct lines, a medium-sized darker band followed by 2 narrow light lines and 2 narrow darker lines in subunit A; B initiates a bulbous section with 2 rather broad dark bands, 3 narrow lines and 3 distinct dark bands; C begins the constriction with a distinct dark band, a clear achromatic space, 2 pairs of narrow bands and a terminal dark band.

Centromere 3. Zone 58 is designated the centromere of the longest chromosome. It holds 2 moderately-sized dark bands (A), 2 lighter bands in an achromatic area that is usually slightly bulbous (B), a constricted area holding a medium band and 2 thin bands (C), followed by 5 light and narrow bands in D.

Left arm of Chromosome 3: Zone 59 holds 2 narrow dark bands, 1 light band, and another broader dark band in A, another 2 dark bands followed by 2 light ones in B, and 2 broad dark bands followed by a single narrow light band in C. Area 60A has a series of closely aligned bands which appear as a broad band, followed by a prominent single band; 60B holds a granulated band followed by 6 solid narrow bands; 60C begins with a broad dark band followed by at least 5 narrow lines. Zone 61A holds 2 single narrow bands flanking an achromatic bulb; B has 2 narrow bands, a broader dark band and again 2 narrow bands, while subunit C has a series of 6 evenly-spaced bands. Zone 62A consists of 2 series of paired narrow bands; B holds a series of closely aligned narrow bands forming a broad dark mass, and C holds 6 narrow evenly-spaced bands. In zone 63, 5 narrow bands comprise area A, B holds a series of 6 narrow bands, and C begins with a dark band, followed by 4 light ones, then darker bands flank a narrow pale marking. Zone 64 has 2 distinct dark bands and 3 narrow faintly-stained lines in A, B has 2 moderately-wide dark bands and a similar 3rd one separated by 2 narrow faint bands. Section 65 holds a single narrow band in an achromatic area in A, two distinct bands flanking a narrow one, followed by another narrow band in B, and C holds a distinct band, a series of 5 narrow light ones, and ends with 2 prominent bands. Zone 66 is highly achromatic holding 4 narrow bands through A, followed by a light, a heavier-broad, then a light, and again heavier band in B.

In zone 67 the pattern is 5 narrow bands ending with a darker broad band in A, B has a granulated band on either side of 2 broader dark bands, and C has a series of 4 single narrow bands. Zone 68A has 3 single light bands followed by a heavier one; B is a highly achromatic area holding 2 light narrow bands and a broader dark band, and ending with a single narrow one. Zone 69A has 1 narrow band flanked on either side by a broad band, then 2 narrow bands; B has 2 light bands followed by 2 denser bands, and C has 2 dense bands followed by 2 light ones, again followed by 2 heavier-stained ones. The last zone begins with a pair of bands followed by a single line (70A); 70B has 3 similar bands in size and density, and 70C terminates the club-like ending of the 3rd chromosome with 2 distinct moderately-sized bands.

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